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‘Malvasia nera di Brindisi/Lecce’ grapevine cultivar (*Vitis vinifera* L.) originated from ‘Negroamaro’ and ‘Malvasia bianca lunga’

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Summary

‘Malvasia nera di Brindisi’ and ‘Malvasia nera di Lecce’ are two of the few Malvasias with black berries and belong to the Apulian ampelographic assortment (South Italy). Their presumed synonymy has been recently ascertained with SSR markers and therefore these two black 'Malvasias' can be considered as an unique variety. We discovered that this cultivar is the cross between ‘Malvasia bianca lunga’ *alias* ‘Malvasia del Chianti’ and ‘Negroamaro’ by using 42 nuclear SSR. Both parents belong to the Apulian varietal resources, since centuries. So far, ‘Malvasia nera di Brindisi/Lecce’ origin has been obscure; now we may assert that this cultivar was born right in Apulia. Three sets of chloroplast SSR loci were used to determine the female and the male parent: 6 ccmp loci, already used in previous pedigree studies, 15 ccSSR loci and 2 NTCP loci, derived from tobacco. The second set of loci was sequenced in order to compare the length of the markers with the reference species where they were originally obtained: in 4 cases no microsatellite motives were detected and in other 4 cases the perfect repetition found in tobacco was not maintained in grape. Unfortunately, the three sets of markers failed to show any polymorphism. A detailed comparison of the black Malvasia morphology with its two parents showed a closer similarity to ‘Negroamaro’. Also the anthocyanin profile is in agreement with that of the black parent; its varietal aroma presents interesting levels of free and bound 2-phenylethanol, responsible for rose flavor, and of bound linalool compounds.

K e y w o r d s : anthocyanin, chloroplast SSR, flavor, nuclear SSR, pedigree.

Introduction

Pedigree reconstruction studies with SSR markers display very interesting information on the relationships among grapevine cultivars and explain their chronological order of appearance (Bowers and Meredith 1997, Bowers *et al*. 1999, Vouillamoz *et al*. 2003, Maletić *et al*. 2004, Di Vecchi Staraz *et al.* 2007, Schneider *et al.* 2008, Crespan *et al.* 2008). This kind of research has not only a cultural interest, since it provides new insights into evolution and nature of the present ampelographic assortment, cultivars identity, variability development and it will be useful for further studies on linkage disequilibrium (Barnaud *et al*. 2006).

'Malvasias' belong to a numerous and heterogeneous population of varieties growing in many European countries and their history is an intriguing enigma. In Italy, at the present moment, 'Malvasias' are spread from North to South and 17 'Malvasia' cultivars are registered in the Italian National Catalogue. Among them ‘Malvasia delle Lipari’ and ‘Malvasia di Sardegna’ have been shown to be synonyms of the same cultivar (Crespan *et al*. 2006 a); moreover, ‘Malvasia rosa’ is a pink mutant for the berry color of the white ‘Malvasia di Candia aromatica’ (Fregoni *et al*. 2005).

In Italy there are few Malvasias with black berries, mostly growing in Piedmont (North West Italy): ‘Malvasia di Casorzo’, ‘Malvasia nera lunga’ and ‘Malvasia di Schierano’; other two, ‘Malvasia nera di Brindisi’ and ‘Malvasia nera di Lecce’, contribute to the Salento oenological production (Apulia, Southern Italy). So far the two Apulian Malvasias have been considered different varieties in the official documents, on the basis of the following characters: ‘Malvasia nera di Lecce’ should have a more dense and heavy bunch compared to ‘Malvasia nera di Brindisi’, that should produce aromatic berries. However not all the experts agree with this distinction, since the observed differences may be due to the environment influence on the same genotype instead of being different varieties.

In a recent study we collected ‘Malvasia nera di Brindisi’ and ‘Malvasia nera di Lecce’ accessions from different vineyards in the Salento traditional cultivation area and compared them analyzing eleven SSR loci. These two Malvasias have been shown to be the same cultivar, since they have the same SSR profile (Coletta *et al*. 2006). Additional more recent SSR profiling, performed on new samples from other vineyards in Apulia, confirmed the published results (Gasparro *et al.* 2008); thus these two Malvasias can be considered as a unique variety, hereafter called ‘Malvasia nera di Brindisi/Lecce’ (MnBR/LE).

Comparing a first set of nuclear SSR loci we found that MnBR/LE could be the progeny of ‘Malvasia bianca lunga’ *alias* ‘Malvasia del Chianti’ (MBL) and ‘Negroamaro’ (NA). Actually these three varieties have been sharing the same cultivation area for a very long time. Salento region is a renowned viticultural area, where once vineyards consisted of few predominant varieties (‘Negroamaro’, ‘Mal-

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vasia nera', 'Susumaniello', 'Malvasia bianca' and some others less important). MBL has been growing in Apulia for centuries; NA is considered to have an even longer presence in the same region and it is one of the most important and popular wine varieties of Salento area (Antonacci 2006). MnBR/LE is bound to the same area, since no information about its presence in other Italian places or in other Countries have been found. So we decided to investigate this potential kinship by extending the molecular analyses to 42 nSSR loci and the initial hypothesis has been confirmed.

In order to distinguish the male and the female parent, we performed chloroplast microsatellite markers analysis, since the chloroplasts are inherited in grapevine from the mother (Strefeler *et al*. 1992, Arroyo-Garcia *et al*. 2002). We used 6 ccmp SSR loci from Weising and Gardner (1999), already successfully applied in grapevine for analogous pedigree studies (Crespan *et al.* 2006 b), and other microsatellite markers detected in tobacco: 15 ccSSR loci found by Chung and Staub (2003) and 2 NTCP loci by Bryan *et al*. (1999).

Morphological and chemical analyses were also performed to evaluate similarities and differences about berry flavor composition and anthocyanin profile between MnBR/LE and the two putative parents.

Material and Methods

Plant material: Mn BR/LE, MBL and NA samples came from the Istituto Sperimentale per la Viticoltura (ISV) collections of Conegliano (Treviso) and Turi (Bari), Italy.

Nuclear SSR markers: The same thirty nSSR used in Crespan *et al*. (2006 b) were analysed, plus twelve additional: VMC4c6, VMC2h4, VMC1b11 and VMC4f3 from Vitis Microsatellite Consortium, scu05 (Scott *et al*. 2000), UCH11 (Lefort *et al.* 2002) and VVIb09, VVIi51, VVIp31, VVIp77, VVIp37 and VVIv36 (Merdinoglu *et al.* 2005). The additional loci were singly amplified and analyzed using the same procedure described in Crespan *et al*. 2006 a.

Likelihood ratios of nSSR data: Molecular data diversity was estimated with Sefc and Wagner (1999) Identity 1.0 program, using nSSR profiles of wine and table grape cultivars of Centro di Ricerca per la Viticoltura molecular database at the first 36 loci in Tab. 1. The number of cultivars used ranged from 400 (the maximum imposed by the program) to 61, with a mean of 230 cultivars per locus. Among them, 120 were table and 280 wine varieties; moreover, 160 cultivars were presumably of Italian origin, the others were more or less spread in Europe. nSSR data available for less than 50 different varieties were not used in this computation.

Chloroplast SSR loci analysis: Twenty-three chloroplast microsatellite loci were analyzed by using the consensus primer pairs designed by Weising and Gardner (1999) for 6 ccmp, by Chung and Staub (2003) for 15 ccSSR loci, and by Bryan *et al*. (1999) for 2 NTCP loci. They were singly amplified, using the following conditions. The PCR reaction mixture (25 µl final volume) contained 20 ng total DNA, 10 µl Eppendorf HotMasterMix (2.5 x) and 10 pmoles of each primer. The PCR was performed in an AB 9700 thermal cycler with the following steps: 1 min 30 s at 94 °C; 35 cycler at 94 °C for 30 s, 55° C for 30 s, 65 °C for 30 s; 65 °C for 7 min and a final step of at least 10 min at 8 °C to stop the reaction. Five µl of the PCR product were tested on 2 % agarose gel. On the basis of signal intensity, 0.1-0.2 µl of amplified DNA were used for electrophoresis with a sequencing gel (5 % polyacrylamide, TBE 1 x, urea 7 M). Gel bands were revealed by silver staining, as reported in Crespan and Milani (2001). Gels were visually scored at least twice.

ccSSR sequencing: ccSSR amplification products obtained from MnBR/LE were sequenced by BMR Genomics, official spin-off of the University of Padova (Italy); sample preparation was performed following the instruction given by the service, for allele length determination and microsatellite motif survey.

Ampelographic comparison: The morphological comparison among the three varieties was performed by means of primary and secondary descriptors, as indicated in the frame of the Genres081 project (http://www.genres.de/eccdb/vitis/).

Flavor composition analysis

Free aroma compounds extraction from berry: Three samples of 450 g were collected for each variety, added with 25 µl of BHA in EtOH (12.66 mg·ml^{-1}), homogenized for 5 min and centrifuged at 4,000 g for 3 min at 20 °C. The supernatant, subdivided in two volumes of about 200 ml, was extracted with CH_2Cl_2 (30 ml) in agitation for 1 h at 10 °C, whereas the precipitate was kept in maceration in CH_2Cl_2 (30 ml) for 4 h at 10 °C. The organic fractions, pooled together, dehydrated with Na_2SO_4 and supplied with 2 µl 2-methyl-pentanol, were first concentrated with rotavapor to about 5 ml and then with a nitrogen flux to reach 1 ml volume.

GC-MSD analysis: GC analyses were performed with an Agilent 6890 N gas chromatograph, equipped with split-splitless injector, a capillary column DB-Wax (60 m x 0.25 mm i.d., 0.5 µm film thickness; J&W Scient. Inc.), interfaced with a detector MS Agilent 5973N.

The temperature program was: 40 °C for 5 min with a ramp of 2 °C·min^{-1}, up to 200 °C for 15 min, with 1 s ramp of 1 °C·min^{-1} up to 250 °C, both for qualitative analysis in full scan and quantitative analysis in SIM. The flow speed of carriage gas (He) was 1 ml·min^{-1}. The mass spectra for full scan analysis were registered in the 28-300 m/z interval. Compound identification was done by comparison of their retention times and mass spectra with those of pure standard references.

The Principal Component Analysis (PCA) was carried out using the values of free aroma compounds detected in at least one of the three varieties, with STATISTICA software version 6.0.

Anthocyanin composition analysis: This analysis was performed only on the two colored varieties, NA and MnBR/LE.

Anthocyanin extraction from skins: Three groups of ten berries were collected randomly from

Table 1

Molecular profiles of 'Malvasia nera di Brindisi/Lecce' and its presumed parents, 'Malvasia bianca lunga' and 'Negroamaro' at 42 nSSR loci. Three reference varieties profiles are also provided for easier data comparison

SSR nuclear loci (linkage group)*	Malvasia bianca lunga	Malvasia nera di Brindisi/Lecce	Negroamaro	Moscato bianco	Cabernet Sauvignon	Sultanina
VVMD26 (1)	249:251	251:251	251:251	251:251	249:251	249:251
VVS29 (1)	171:171	171:171	171:171	171:171	179:181	171:179
ISV3 (2)	133:139	133:139	139:145	133:139	133:139	133:139
VVMD28 (3)	251:257	237:251	237:239	249:271	239:237	221:247
VVMD36 (3)	254:254	254:270	244:270	244:264	254:264	250:268
VVIp37 (4)	118:134	118:118	118:138	138:138	150:150	134:138
VVIp77 (4)	173:185	181:185	181:191	189:191	181:185	173:199
VVMD32 (4)	253:257	253:253	253:273	273:265	241:241	251:251
VrZAG 21 (4)	204:206	202:206	202:206	206:206	200:206	190:202
VMC4C6 (5)	160:163	157:163	157:163	157:157	163:175	151:157
VMC6E10 (5)	95: 97	97:117	97:117	111:113	111:121	121:127
VVMD27 (5)	179:179	179:181	179:181	179:194	175:189	181:194
VrZAG 79 (5)	242:250	250:258	258:258	250:254	246:246	246:258
VMC2G2 (6)	119:119	119:119	119:125	123:125	121:125	125:125
VMC2H9 (6)	117:117	117:117	117:123	121:123	119:123	123:123
VMC4G6 (6)	133:143	129:143	129:143	127:143	127:133	125:129
VMCNG4B9 (6)	150:176	150:158	158:168	158:166	168:176	138:158
VVMD21 (6)	249:249	249:249	249:249	249:266	249:258	249:256
VVIv36 (7)	149:155	149:155	149:155	149:153	149:155	155:155
VVMD7 (7)	239:253	239:249	249:249	233:249	239:239	239:253
VVMD31 (7)	212:214	212:212	212:212	212:216	206:210	212:212
VrZAG 62 (7)	195:199	195:201	189:201	185:195	187:193	187:187
VMC1B11 (8)	174:198	168:174	168:172	186:190	186:186	168:186
VrZAG 83 (8)	190:194	194:194	188:194	188:188	200:200	188:194
VMC3D7 (10)	163:171	163:163	159:163	161:163	159:161	161:163
VrZAG 64 (10)	137:159	143:159	137:143	141:159	139:159	143:159
ISV4 (11)	177:177	177:187	169:187	169:187	169:191	191:193
VVMD25 (11)	243:245	245:245	245:267	245:253	243:253	243:253
VVS2 (11)	145:145	145:145	145:151	133:133	139:151	145:151
VMC2H4 (12)	198:200	200:214	206:214	200:214	212:220	204:214
scu 05 (12)	163:165	163:165	165:169	160:165	165:168	165:171
ISV2 (14)	143:165	143:143	141:143	141:143	141:165	143:143
VMC1E12 (14)	250:260	250:260	250:250	260:260	240:250	244:260
VVMD24 (14)	210:210	210:214	214:214	214:219	210:219	210:219
VVMD5 (16)	226:240	226:236	226:236	228:236	232:240	234:234
VVIb09 (17)	275:279	269:275	269:279	269:279	275:277	277:279
VVMD17 (18)	221:222	222:222	222:222	220:222	221:222	222:222
VVIp31 (19)	171:175	171:187	181:187	181:185	187:187	177:181
VVS1 (20)	180:181	181:190	181:190	181:181	181:181	181:188
UCH11	242:262	242:248	244:248	244:248	244:262	242:244
VMC4F3	168:206	168:168	168:168	168:206	174:180	190:192
VVIi51	246:260	246:248	246:248	246:260	260:260	246:248

* The linkage group numbers, where available, refer to Riaz *et al*. (2004) reference map.

5 clusters for each cultivar, weighed and peeled. After a brief drying, skins were soaked in 25 ml ethanol hydrochloric and kept in infusion for at least 12 h. The extract was separated from skins by settling, filtered with regenerated cellulose filters of 0.45 μm and directly analyzed by HPLC.

HPLC-DAD analysis: The chromatographic separation was done on Zorbax SB-C_{18} Agilent (4,6 x 250 mm, 5 μm) supplied with a "guard column" Phenomenex; the injection volume was 5 μl. For mobile phase, acetonitrile (solvent A) and formic acid 10 % (solvent B) were used. The elution program provided a constant flow of 0.7 ml·min^{-1} and a gradient: 0 min, 5 % A-95 % B; 10 min, 13 % A-87 % B; 20 min, 15 % A-85 % B; 30 min, 22 % A-78 % B; 50 min, 22 % A-78 % B; 55 min, 5 % A-95 % B. Rebalancing period for next sample charge was 10 min with the solvent mixture used at time zero.

The analysis was performed by monitoring the absorbance signal at 520 nm. The peaks identification was obtained by comparison of retention times and absorbance spectra of analogous compounds reported in literature (García-Beneytez *et al*. 2003) with those of pure chemicals.

Results and Discussion

Nuclear SSR data: Among the nSSR loci used, only three are not yet mapped and the others are spread over all chromosomes except LG9, LG13 and LG15. This choice was suggested by the opportunity to validate the possible first degree relationship with markers independently inherited, therefore more informative than linked markers. MnBR/LE exhibited one allele derived from each of the presumed parents at all fourty-two nuclear loci, supporting the preliminary hypothesis about a parent-progeny relationship among these cultivars (Tab. 1).

Cumulative likelihood ratios of MnBR/LE being the progeny of MBL (1) and NA (2), *versus* alternative parents, including close relatives are reported in Tab. 2. These estimates showed that the indicated parents were more than 10^{21} times more probable than other combinations of two

Table 2

Cumulative likelihood ratios of 'Malvasia nera di Brindisi/Lecce' being the progeny of 'Malvasia bianca lunga' (1) and 'Negroamaro' (2), versus alternative parents, including close relatives, combined over 36 nuclear SSR loci

Parents combinations	1 x 2	(1) x X	rel (2) x (1)	(2) x X	rel (1) x (2)
with observed allele frequencies	$3{,}49 \times 10^{21}$	$3{,}74 \times 10^{14}$	$1{,}01 \times 10^{5}$	$2{,}88 \times 10^{11}$	$8{,}62 \times 10^{3}$
with 95 % upper confidence limit	$8{,}01 \times 10^{16}$	1.25×10^{12}	$2{,}04 \times 10^{4}$	$1{,}52 \times 10^{9}$	$1{,}59 \times 10^{3}$

random cultivars (more than 10^{16} with 95 % upper confidence limit of allele frequencies). These ratios remained far higher also compared to the values calculated when one of the suggested parents was assumed and the other parent was a close relative of the second suggested parent.

Chloroplast SSR data: Three sets of chloroplast SSR loci were used to determine the sexual role of each parent of MnBR/LE. The 6 ccmp chloroplast microsatellites did not show any polymorphism among the three varieties and their haplotype was the same as 'Raboso Piave' and 'Raboso veronese' (CRESPAN *et al*. 2006 b). A set of 15 ccSSR loci, designed by CHUNG and STAUB (2003), was used in the second analysis. In this case all primer pairs gave a strong amplification product between 180 and 360 bp, nevertheless none detecting length differences. An analysis of ccSSR allele size was performed by sequencing of MnBR/LE amplicons and comparison with those obtained in tobacco, as reported in Tab. 3. In four cases no microsatellite motives were identified (ccSSR12, ccSSR15, ccSSR18 and ccSSR22), in other cases the perfect repetition found in tobacco was interrupted in grape (ccSSR9, ccSSR17, ccSSR19 and ccSSR21); in general, the repeated stretches were rather short and this could be the reason for length polymorphism absence in the grape cultivars analyzed in this study.

NTCP8 and NTCP12 loci (BRYAN *et al.* 1999) were also analysed. Only the first one, identified in the Solanaceae family, amplified efficiently in grapevine and showed to be polymorphic in a preliminary screening on a limited number of varieties, but without useful results in our three samples. As NTCP8 PCR product was not sequenced, its length was estimated around 250 bp, with a 100 bp ladder on a 2 % agarose gel. In summary, the three cultivars showed the same haplotype and the absence of any polymorphism did not allow to define the male and the female parent.

Ampelographic comparison: MnBR/LE showed a very higher resemblance with NA than MBL, sharing as many as 24 characters exclusively with the black parent, but only 10 with MBL (Tab. 4). On the whole, however, the morphological similarity of MnBR/LE came up with both parents, since as many as 23 characters were in common with the same expression level (Tab. 5). Only 8 characters showed expression levels different from those of the two parents (Tab. 6).

Chemical berry composition comparison: In this study some secondary metabolites, aromatic and anthocyanin compounds, were quantified due to their importance for cultivar characterization.

Free aroma compounds: Aromatic compounds of MBL (STORCHI *et al*. 2005) were compared with those of NA and MnBR/LE obtained from the analysis of clusters coming from Turi (Bari, Italy). The total amount of lipid derivatives was highly similar between the two black varieties. Benzyl compounds had a higher concentration, from 4 to 6 times, in the offspring than in the two parents. Only MnBR/LE showed high levels of free monoterpenols (160 ± 30 µg/kg): we found the relevant presence of *cis*-furan-linalool-oxide and *trans*-furan-linalool-oxide, not detectable in the two parents, neither as traces. On the contrary, HO-trienol and geraniol were not detected in the black Malvasia, even if they were present as traces in NA and in MBL. It was noteworthy the absence of many terpene compounds, such as linalool, nerol and α-terpineol, in the three cultivars studied, which is a common trend for non aromatic varieties (BORSA *et al*. 2005).

The PCA analysis was carried out with the 14 compounds listed in Tab. 7. It allowed to find three well defined

Table 3

Chloroplastic SSR allele length (bp) in grape compared with tobacco (after CHUNG and STAUB 2003) and microsatellite motif found in 'Malvasia nera di Brindisi/Lecce'

Locus name	SSR motif in grape	SSR motif in tobacco	expected size in tobacco	size in *Vitis vinifera* L.
ccSSR4	(T)8	(T)8	205	279
ccSSR6	(T)10	(T)8	299	299
ccSSR7	(T)11	(T)11	349	359
ccSSR9	(A)3T(A)10	(A)13	173	167
ccSSR12	no microsatellite motives found	(A)8	249	236
ccSSR13	(T)8	(T)9	264	279
ccSSR15	no microsatellite motives found	(T)9	264	264
ccSSR16	(T)11	(T)7C(T)2	123	356
ccSSR17	(A)5G(A)7	(A)13	236	227
ccSSR18	no microsatellite motives found	(A)8	264	264
ccSSR19	(T)3C(T)7	(T)8	335	359
ccSSR20	(A)13	(A)8	311	329
ccSSR21	(A)11 - (T)7C(T)5	(T)13	280	281
ccSSR22	no microsatellite motives found	(T)8	190	185
ccSSR23	(A)7 - (A)15 - (A)9	(A)14	217	281

Table 4

'Malvasia nera di Brindisi/Lecce' characters in common only with one parent

Negroamaro			Malvasia bianca lunga		
Character code	Description	Expression level	Character code	Description	Expression level
3	Young shoot: intensity of anthocyanin coloration on prostrate hairs of the tip	7	76	Mature leaf: shape of teeth	5
8	Shoot: color of ventral side of internodes	2	452	Leaf: degree of resistance to Plasmopara	3
015_2	Shoot: intensity of anthocyanin coloration on the bud scales	5	606	Mature leaf: length petiole sinus to lower leaf sinus	3
74	Mature leaf: profile	3	613	Mature leaf: width of teeth N2	5
79	Mature leaf: opening/overlapping of petiole sinus	2	459	Cluster: degree of resistance to Botrytis	5
455	Leaf: degree of resistance to Oidium	7	223	Berry shape	5
601	Mature leaf: length of vein N1	5	236	Berry particular flavor	5
602	Mature leaf: length of vein N2	5	617	Mature leaf: length between the tooth tip of N2 and the tooth tip of the first secondary vein of N2	7
066_5	Mature leaf: vein N3, length petiole sinus to vein N4	5	066_4	Mature leaf length of vein N5	3
87	Mature leaf: density of erect hairs on the main veins (lower side)	5	84	Mature leaf: density of prostrate hairs beween the main veins (lower side)	5
079_1	Mature leaf: opening/overlapping of petiole sinus	1			
202	Bunch: length	5			
206	Bunch: length of peduncle	1			
208	Bunch: shape	1			
209	Bunch: number of wings	3			
609	Mature leaf: angle between N3 and N4	3			
615	Mature leaf: width of teeth N4	3			
616	Mature leaf: number of teeth between the tooth tip of N2 and the tooth tip of the first secondary vein of N2 including the limits	9			
220	Berry: length	5			
225	Berry: color of skin	6			
502	Bunch: weight of a single bunch	3			
503	Berry: single berry weight	5			
505	Sugar content of must	9			
508	pH of must	3			

Table 5

'Malvasia nera di Brindisi/Lecce' characters in common with both parents

Character code	Description	Expression level
1	Young shoot: shape of the tip	7
4	Young shoot: density of prostrate hairs of the tip	7
6	Shoot: attitude	7
16	Tendrils: distribution on the shoot	2
70	Mature leaf: anthocyanin coloration of the main veins on the upper side of the blade	1
72	Mature leaf: goffering of blade	1
603	Mature leaf: length of vein N3	5
604	Mature leaf: length of vein N4	7
605	Mature leaf: length petiole sinus to upper leaf sinus	3
612	Mature leaf: length of teeth N2	3
614	Mature leaf: length of teeth N4	3
51	Young leaf: color of the upper side (leaf 4)	1
53	Young leaf: density of prostrate hairs between veins at the lower side of leaf	7
80	Mature leaf: shape of base of petiole sinus	1
081_1	Mature leaf: presence of teeth in the petiole sinus	1
081_2	Mature leaf: petiole sinus limited by veins	1
083_2	Mature leaf: presence of teeth at the base of the upper leaf sinuses	1
67	Mature leaf: shape of blade	3
68	Mature leaf: number of lobes	3
204	Bunch: density	7
230	Berry: color of flesh	1
235	Berry: degree of firmness of flesh	5
241	Berry: presence of seeds	3

and no overlapping groups (Fig. 1), one for each variety. The variables giving a very high contribution to the first component, that explains 50.79 % of total data set variance, are n-hexanol, hexanoic acid, benzyl alcohol, 2-phenyl-ethanol, *cis*- and *trans*-linalool-oxide. The second principal component (40.94 % of total variability) was influenced by *trans*-2-hexenal, capronaldehyde, *trans*-2-hexenol, *cis*-3-hexenol, HO-trienol and geraniol.

Table 6

'Malvasia nera di Brindisi/Lecce' characters different from both parents

Character code	Description	Malvasia bianca lunga	Malvasia nera di Brindisi/ Lecce	Negroamaro
		Expression level		
7	Shoot: color of dorsal side of internodes	1	3	2
015_1	Shoot: distribution of the anthocyanin coloration on the bud scales	1	9	5
75	Mature leaf: blistering of upper side	5	3	5
607	Mature leaf:angle between N1 and N2 measured at the first ramification	9	3	5
608	Mature leaf:angle between N2 and N3 measured at the first ramification	7	3	5
610	Mature leaf:angle between N3 and the tangent between petiol point and the tooth tip of N5	9	7	9
221	Berry width	3	5	3
506	Total acid content of must	1	3	7

Table 7

Free aroma compounds used for Principal Component Analysis and factor coordinates of these variables

Variables	Factor 1	Factor 2
trans-2-Hexenal		0,927606
Capron-aldehyde		0,976376
n-Hexanol	-0,997165	
trans-2-Hexenol		-0,820160
cis-3-Hexenol		-0,964508
Hexanoic acid	-0,938463	
Benzyl-alcohol	-0,973926	
Phenyl-acetaldehyde		0,769587
Benzyl-aldehyde		0,61868
2-Phenyl-ethanol	-0,994165	
cis-Furan-linalool-oxide	-0,986958	
trans-Furan-linalool-oxide	-0,978152	
Ho-trienol		0,778596
Geraniol		-0,802948

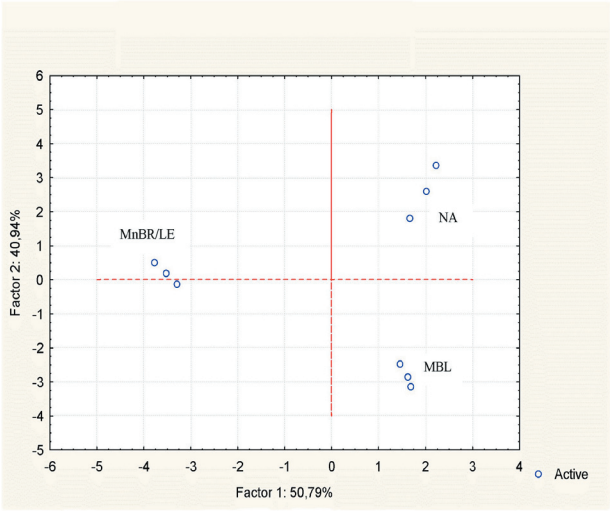

Fig. 1: Principal component diagram of 'Malvasia bianca lunga' (MBL), 'Malvasia nera di Brindisi/Lecce' (MnBR/LE) and 'Negroamaro' (NA) as a function of 14 variables for 9 must samples. Factor score plot 1-2: axis 1 and 2 account for 91.7 % of the total variance.

NA musts were characterized by compounds associated with positive values of the two first principal components: *trans*-2-hexenal, capronaldehyde and HO-trienol; MBL musts were characterized, primarily, by *cis*-3-hexenol and geraniol; finally, MnBR/LE samples by n-hexanol, benzyl alcohol, 2-phenyl-ethanol, *cis*- and *trans*-linalool-oxide.

Bound aroma compounds: Benzyl and terpene compounds were examined by putting together the data produced by Di Stefano *et al*. (1997) and Borsa *et al*. (2005); their results were reported in Tab. 8. The total bound benzyl aromas of black Malvasia had a concentration in-between the two parents and closer to NA. Interestingly, *2-phenyl-ethanol* level was very similar to that of the white Malvasia. The same intermediate trend was shown by terpene compounds quantities. As NA, the black Malvasia was characterized by a varietal terpene aroma, with compounds belonging mainly to the linalool class.

Anthocyanin characterization: The total amount of anthocyanins showed that MnBR/LE produced about half color than NA, 319 and 639 $mg \cdot kg^{-1}$ respectively. The relative percentage of each pigment, and therefore the anthocyanin profile, was almost the same in both cultivars (Fig. 2).

Conclusions

We showed that MnBR/LE could be the progeny of MBL and NA. Molecular data and available information on the historical distribution of MnBR/LE led to the conclusion that this variety originated in Apulia, where it found a suitable environment for its success. Therefore it was not imported in this region from Greece or from other East Mediterranean Countries, as generally hypothesized for Malvasia varieties (Galet 2000, Calò *et al*. 2001).

Our study proved that transferability of chloroplast markers from plant species very distant from grape is also possible, but their SSR polymorphism may be very low, or absent. Moreover, the repeated motif found in the reference species may be lacking in the new one analyzed. For this reason it is difficult to identify the sexual role of each parent in spontaneous plant breeding, as in the case reported here. MnBR/LE shows a greater resemblance with NA than with MBL, and actually it is likely to confuse the two varieties. Analogous cases, now explainable with parent-offspring relationship, are well known: for example, the 'Chardonnay' is morphologically very similar to the white phenotype of the parent 'Pinot' and in Italy they have been confused for long time; the same difficulty is found in distinguishing 'Raboso veronese' from its mother, 'Raboso Piave'.

Table 8

Concentration of the glycosidically bound aroma compounds released by enzymatic hydrolysis. Values are expressed as μg·kg^{-1} of berry fresh weight

Bound aromatic compounds		Negroamaro	Malvasia nera di Brindisi/Lecce	Malvasia bianca lunga
Benzilic compounds	Benzylalcohol	232	n.d.	n.d.
	Acetovanillone	24	130	37
	Zingerone	29	26	4
	Vanillin	5	14	26
	2-Phenyl-ethanol	166	239	235
	Total	456	409	302
Terpenes	*cis*-Furan-linalool-oxide	3	29	4
	trans- Furan-linalool-oxide	12	33	4
	trans-8-OH-linalool	43	92	18
	cis-8-OH-linalool	124	51	74
	Linalool	20	9	9
	Geraniol	39	8	24
	Geranic acid	6	22	11
	Nerol	7	4	6
	α-Terpineol	3	3	65
	Total	257	251	215

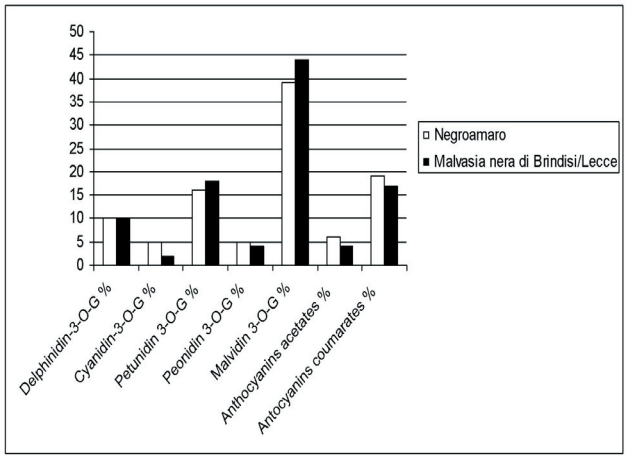

Fig. 2: Anthocyanic profile of 'Negroamaro' and 'Malvasia nera di Brindisi/Lecce'.

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References

Antonacci, D.; 2006: Grapevines of Apulia. Mario Adda Editor, Bari.

Arroyo-García, R.; Lefort, F.; De Andrés, M. T.; Ibañez, J.; Borrego, J.; Jouve, N.; Cabello, F.; Martínez-Zapater, J. M.; 2002: Chloroplast microsatellite polymorphisms in *Vitis* species. Genome **45**, 1142-1149.

Barnaud, A.; Lacombe, T.; Doligez, A.; 2006: Linkage disequilibrium in cultivated grapevine, *Vitis vinifera* L. Theor. Appl. Genet. **112**, 708-716.

Borsa, D.; Carniel, D.; Asproudi, A.; Monticelli, L.; Crespan, M.; Costacurta, A.; 2005: Characterization of Malvasia grapes by secondary metabolites. Riv. Vitic. Enol. **2-3-4**, 167-182.

Bowers, J. E.; Meredith, C. P.; 1997: The parentage of a classic wine grape, Cabernet Sauvignon. Nat. Genet. **16**, 84-87.

Bowers, J. E.; Boursiquot, J. M.; This, P.; Chu, K.; Johansson, H.; Meredith, C.; 1999: Historical genetics: The parentage of Chardonnay, Gamay, and other wine grapes of Northeastern France. Science **285**, 1562-1565.

Bryan, G. J.; McNicoll, J.; Ramsay, G.; Meyer, R. C.; De Jong, W. S.; 1999: Polymorphic simple sequence repeat markers in chloroplast genomes of Solanaceous plants. Theor. Appl. Genet. **99**, 859-867.

Calò, A.; Costacurta, A.; Scienza, A.; 2001: Le principali famiglie varietali italiane. In: Vitigni d'Italia. Ed. Calderini Edagricole.

Chung, S. M.; Staub, J. E.; 2003: The development and evaluation of consensus chloroplast primer pairs that possess highly variable sequence regions in a diverse array of plant taxa. Theor. Appl. Genet. **107**, 757-767.

Coletta, A.; Crespan, M.; Costacurta, A.; Caputo, A. R.; Taurisano, C.; Meneghetti, S.; Antonacci, D.; 2006: Preliminary investigations on Malvasia nera di Lecce and Malvasia nera di Brindisi varieties. Riv. Vitic. Enol. **2-3**, 51-56.

Crespan, M.; Milani, N.; 2001: The Muscats: A molecular analysis of synonyms, homonyms and genetic relationships within a large family of grapevine cultivars. Vitis **40**, 23-30.

Crespan, M.; Cabello, F.; Giannetto, S.; Ibáñez, J.; Kontić, J. K.; Maletić, E.; Pejić, I.; Rodriguez, I.; Antonacci, D.; 2006 a: Malvasia delle Lipari, Malvasia di Sardegna, Greco di Gerace, Malvasia de Sitges and Malvasia dubrovačka – synonyms of an old and famous grape cultivar. Vitis **45**, 69-73.

Crespan, M.; Cancellier, S.; Chies, R.; Giannetto, S.; Meneghetti, S.; 2006 b: The parents of Raboso veronese were discovered: a new hypothesis on its origin. Riv. Vitic. Enol. **1**, 3-12.

Crespan, M.; Calò, A.; Giannetto, S.; Sparacio, A.; Storchi, P.; Costacurta, A.; (2008): 'Sangiovese' and 'Garganega' are two key varieties of the Italian grapevine assortment evolution. Vitis **47**, 97-104.

Di Stefano, R.; Pigella, R.; Gentilini, N.; Calò, A.; Costacurta, A.; 1996-1997: Il "Negroamaro", vitigno dalle molteplici possibilità enologiche. Annali dell'Istituto Sperimentale per l'Enologia Asti (Italy) **27**, 149-163.

Di Vecchi Staraz, M.; Bandinelli, R.; Boselli, M.; This, P.; Boursiquot, J. M.; Laucou, V.; Lacombe, T.; Varès, D.; 2007: Genetic structuring and parentage analysis for evolutionary studies in grapevine: Kin group and origin of the cultivar Sangiovese revealed. J. Am. Soc. Hortic. Sci. **132**, 514-524.

Fregoni, M.; Civardi, S.; Vercesi, A.; Zamboni, M.; 2005: Malvasia rosa: a new aromatic grapevine variety by bud mutation. Riv. Vitic. Enol. **2-3-4**, 39-44.

Galet, P.; 2000: Dictionnaire Encyclopédique des Cépages. Ed. Hachette.

García-Beneytez, E.; Cabello, F.; Revilla, E.; 2003: Analysis of grape and wine anthocyanins by HPLC-MS. J. Agric. Food Chem. **51**, 5622-5629.

Gasparro, M.; Caputo, A. R.; Brini, M. L.; Coletta, A.; Crespan, M.; La Penna, F.; Antonacci, D.; 2008: Caratterizzazione di accessioni di Malvasia nera con il metodo dei marcatori molecolari. In the Proceedings, available as CD, of the 31st World Congress of vine and wine, June 15-20, Verona, Italy.

Lefort, F.; Kyvelos, C. J.; Zervou, M.; Edwards, K. J.; Roubelakis-Angelakis, K. A.; 2002: Characterization of new microsatellite loci from *Vitis vinifera* and their conservation in some *Vitis* species and hydrids. Mol. Ecol. Notes **2**, 20-21.

Maletić, E.; Pejić, I.; Kontić, J. K.; Piljac, J.; Dangl, G. S.; Vokurka, A.; Lacombe, T.; Mirošević, N.; Meredith, C. P.; 2004: Zinfandel, Dobričič, and Plavac mali: the genetic relationship among three cultivars of the Dalmatian coast of Croatia. Am. J. Enol. Vitic. **55**, 174-180.

Merdinoglu, D.; Butterlin, G.; Bevilacqua, L.; Chiquet, V.; Adam-Blondon, A. F.; Decroocq, S.; 2005: Development and characterization of a large set of microsatellite markers in grapevine (*Vitis vinifera* L.) suitable for multiplex PCR. Mol. Breed. **15**, 349-366.

Riaz, S.; Dangl, G. S.; Edwards, K. J.; Meredith, C. P.; 2004: A microsatellite based framework linkage map of *Vitis vinifera* L. Theor. Appl. Genet. **108,** 864-872.

Schneider, A.; Torello Marinoni, D.; Crespan, M.; 2008: Genetics and ampelography trace the origin of Muscat fleur d'oranger. Am. J. Enol. Vitic. **59**, 200-204.

Scott, K. D.; Eggler, P.; Seaton, G.; Rossetto, M.; Ablett, E. M.; Lee, L. S.; Henry, R. J.; 2000: Analysis of SSRs derived from grape ESTs. Theor. Appl. Genet. **100**, 723-726.

Storchi, P.; Armanni, A. B.; Meneghetti, S.; Borsa, D.; Monticelli, L.; 2005: Recovery and exploitation of an ancient Malvasia of Tuscany biotype: the "Malvagia of Montegonzi". Riv. Vitic. Enol. **2-3-4**, 13-24.

Strefeler, M. S.; Weeden, N. F.; Reisch, B. I.; 1992: Inheritance of chloroplast DNA in two full-sib *Vitis* populations. Vitis **31**, 183-187.

Vouillamoz, J.; Maigre, D.; Meredith, C. P.; 2003: Microsatellite analysis of ancient alpine grape cultivars: Pedigree reconstruction of *Vitis vinifera* L. 'Cornalin du Valais'. Theor. Appl. Genet. **107**, 448-454.

Vouillamoz, J. F.; Monaco, A.; Costantini, L.; Stefanini, M.; Scienza, A.; Grando, M. S.; 2007: The parentage of "Sangiovese", the most important italian wine grape. Vitis **46**, 19-22.

Weising, K.; Gardner, R.; 1999: A set of conserved PCR primers for the analysis of simple sequence repeat polymorphism in chloroplast genomes of dicotyledonous angiosperms. Genome **42,** 9-19.

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